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<b>13. ABSTRACT (Maximum 200 Words)</b> <p>Neoadjuvant chemotherapy is currently the standard of care for locally advanced breast cancer (LABC). Monitoring tumor response is advantageous for patients. This project aims to using the subharmonic signals from ultrasound contrast agents to improve the monitoring of breast cancer treatment response to neoadjuvant therapies in women diagnosed with LABC by imaging tumor angiogenesis with 3D subharmonic imaging (SHI) and by estimating the interstitial fluid pressure (IFP) using 3D subharmonic aided pressure estimation (SHAPE).</p> <p>To date, software for analyzing RF data from a Logiq 9 ultrasound scanner (GE Healthcare, Milwaukee, WI) to produce 3D SHAPE pressure estimates has been successfully developed. Initial <i>in vivo</i> experiments in 2 canines have been completed. SHI and the optimization algorithm implemented indicate that the use of SHAPE for noninvasive evaluation of the IFP in breast lesions is feasible with Definity. Moreover, However, difficulty in obtaining the necessary approvals for our human clinical trial has delayed the project by approximately 8 months.</p>				
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## 4 INTRODUCTION

In the United States, close to 5 – 20 % of newly diagnosed breast cancer and 10 – 30% of all primary breast cancer is diagnosed as locally advanced breast cancer (LABC) [1, 2]. Neoadjuvant chemotherapy (systemic preoperative chemotherapy) is currently the standard of care for LABC [3, 4]. When compared with adjuvant chemotherapy (postoperative therapy), neoadjuvant chemotherapy yields similar results for both overall survival (70% for both) and disease-free survival (53% adjuvant, 55% neoadjuvant) [5]. Thus, the postponement of surgery does not affect the outcome of the treatment [5, 6]. In addition, neoadjuvant chemotherapy offers considerable benefits to the patient as the treatment can shrink the tumor and even in some cases offer complete pathologic response [3, 7]. This reduction in tumor size increases the possibility of breast conservation [3, 5-7]. Maximizing the conservation of breast tissue can be of great personal importance for the self-esteem and quality of living of the patient [6]. Neoadjuvant chemotherapy can also offer an early indication of the patient's response to chemotherapy. Consequently, monitoring tumor response to neoadjuvant therapy gives the possibility of adjusting the treatment if the patient is responding poorly or not at all resulting in substantial advantages for the patient [3, 6]. This project aims at establishing noninvasive monitoring of neoadjuvant chemotherapy in the breast using subharmonic aided pressure estimation (SHAPE; U.S. Patent 6,302,845).

Generally interstitial fluid pressure (IFP) is 10-30 mmHg higher in cancerous tissue than in normal tissue although values of up to 60 mmHg have been recorded [8, 9]. Similarly, IFP in breast cancer tumors has been shown to be higher than that of surrounding breast tissue [9]. This increase is believed to be due to vascularity, fibrosis and difference in the interstitial matrix in tumors and it can result in poor transport of therapeutic drugs to tumors [8]. Taghian et al. used a wick-in-needle technique to monitor the IFP of breast cancer before and after neoadjuvant chemotherapy with two drugs used consecutively [10]. When used as a first drug Paclitaxel decreased the IFP by 36% ( $p=0.02$ ) whereas with Doxorubicin as a first drug there was only 8% reduction ( $p=0.41$ ). As this was a hypothesis-generating study they did not show any outcome related to the relationship between IFP and therapy response [10]. However, the level of IFP has been shown to predict disease free survival for cervix cancer (34% disease free survival (DFS) if  $IFP > 19$  mmHg, 68% DFS if  $IFP < 19$  mmHg ( $p = 0.002$ )) [11]. Thus, the level of interstitial fluid pressure (IFP) in breast cancer tumors could potentially be used to monitor the response to neoadjuvant chemotherapy.

Contrast agents have been used for two decades to improve visualization in ultrasound (US) imaging as they enhance the difference in reflectivity between tissues [12]. Because of the difference in compressibility between the medium and the microbubble any changes in pressure induce changes in the size of the microbubble [13]. This in turn affects the reflectivity and resonant frequency of the bubble [13, 14]. In subharmonic imaging (SHI) pulses are transmitted at a frequency  $f_0$  and the echoes are received at half that frequency  $f_0/2$ . SHI has been showed to be a feasible option for contrast enhanced imaging due to subharmonic generation by contrast agents and limited subharmonic generation in tissues [15]. Our group came up with a novel technique, SHAPE, utilizing

microbubbles and the subharmonic amplitude of the scattered signal [13]. We showed that there is a linear relationship between the hydrostatic pressure and the subharmonic amplitude. We propose the use of SHAPE to monitor treatment response by noninvasively measuring the IFP in breast tumors. This offers several benefits to the patient. As opposed to the wick-in-needle technique SHAPE is noninvasive and does not inflict pain. Furthermore, it allows for an early indication of responders vs. non-responders and thereby makes adjustments to therapy easier. Moreover, SHAPE has been shown to have a favorable signal-to-noise ratio so the subharmonic amplitude is not affected by background noise [13].

The SHAPE algorithm will be implemented on a state-of-the-art ultrasound scanner (Logiq 9, GE Healthcare, Milwaukee, WI) for *in vivo* monitoring of angiogenesis and IFP. This implementation will be tested with the 4D10L 3D probe and optimized *in vivo* (in canines) with the contrast agent Definity. The ability of SHI to depict macro- and micro-vascularity (the latter as a model for tumor angiogenesis) will also be assessed. Finally, the ability of 3D SHI and SHAPE to monitor neoadjuvant chemotherapy in women with LABC (i.e., the ability to track changes in tumor angiogenesis and IFP, respectively) will be evaluated in a first in humans clinical trial. We plan to recruit 10 – 25 subjects per year who will be studied with contrast US (SHI and SHAPE) before as well as after the 1<sup>st</sup>, 3<sup>rd</sup> and last chemotherapy cycle. All subjects will also receive an MRI as part of their standard of care (before and after completion of the chemotherapy), and these results together with pathology will be compared to the 3D SHI and SHAPE studies (independently as well as combined) as a means for treatment monitoring.

Our group has proposed that SHAPE and contrast enhanced US imaging can be used to measure the IFP in LABC tumors, thus, making it possible to noninvasively monitor the tumor response to neoadjuvant chemotherapy. This method would be a considerable improvement from the wick-in-needle technique currently used for IFP measurements in LABC and allow for individualized treatments options.

## 5 BODY

The hypothesis of this project is that IFP in breast tumors can be measured noninvasively using SHAPE and contrast enhanced US thus improving the monitoring of neoadjuvant chemotherapy. First 3D SHI and SHAPE will be implemented on a commercial scanner. The scanner will be used to monitor neoadjuvant chemotherapy in women with LABC in a first in humans clinical trial. We plan to recruit 20 – 50 subjects who will be studied with contrast SHI and SHAPE before as well as after the 1<sup>st</sup>, 3<sup>rd</sup> and last chemotherapy cycle. The specific tasks of the project (as presented in the original Statement of Work) can be found in Appendix I.

First an outline of the methods applied will be given followed by a presentation of the results to date. Finally, the conclusions and future directions of the research will be discussed.

## 5.1 Methods

Obtaining regulatory approval for the animal as well as human subjects components of this proposal was initiated in July 2012 and by August we submitted for approval of our animal optimization studies to the Thomas Jefferson University (TJU) Institutional Animal Care and Use Committee (IACUC). We received approval by the IACUC at the end of August and on September 17<sup>th</sup>, 2012 our protocol was submitted to the USAMRMC Animal Care and Use Review Office (ACURO). This protocol was approved by the USAMRMC ACURO in end October/early November of 2012.

Concurrently with the software developments detailed below, we also submitted our protocol to Lantheus Medical Imaging for Definity on December 6<sup>th</sup>, 2012. Next, we prepared our submission to the Food and Drug Administration (FDA) for an Investigational New Drug number. However, after discussion with the FDA we determined that an amendment to our existing IND application (#112,241) would be sufficient. This was submitted on February 26<sup>th</sup>, 2013 and approved by the FDA without comments 30 days later (i.e., March 25<sup>th</sup>). While the FDA were reviewing our protocol, we prepared the required documentation for the TJU Clinical Cancer Research Review Committee (CCRRC), which is an initial human subjects' approval committee mandated by the National Cancer Institute (NCI), because of the NCI designated Cancer Center at TJU. The CCRRC received our protocol submission on March 25<sup>th</sup> and following approval in April of 2013, we progressed to the TJU Institutional Review Board (IRB), which provided the final TJU required human subjects approval on June 6<sup>th</sup>, 2013. Then we prepared documentation to be in compliance with the USAMRMC human subjects protection requirements under the Office of Research Protections (ORP). The ORP provides human subjects protection review and compliance oversight under the Human Research Protections Office (HRPO), which received our submission on June 17<sup>th</sup>, 2013. After having received several ORP HRPO reviews during the summer and made the appropriate amendments (which had to be approved by the TJU IRB), we submitted the (presumed) final version of our protocol for approval by the ORP HRPO on October 30<sup>th</sup>, 2013 and we are currently awaiting a response.

Given the delay caused by the efforts required to obtain regulatory approval for the animal and human clinical trials, the project is approximately 8 months behind schedule and we, therefore, intend to request a one year no cost extension to complete the SOW (Appendix I).

### *In vivo experiments*

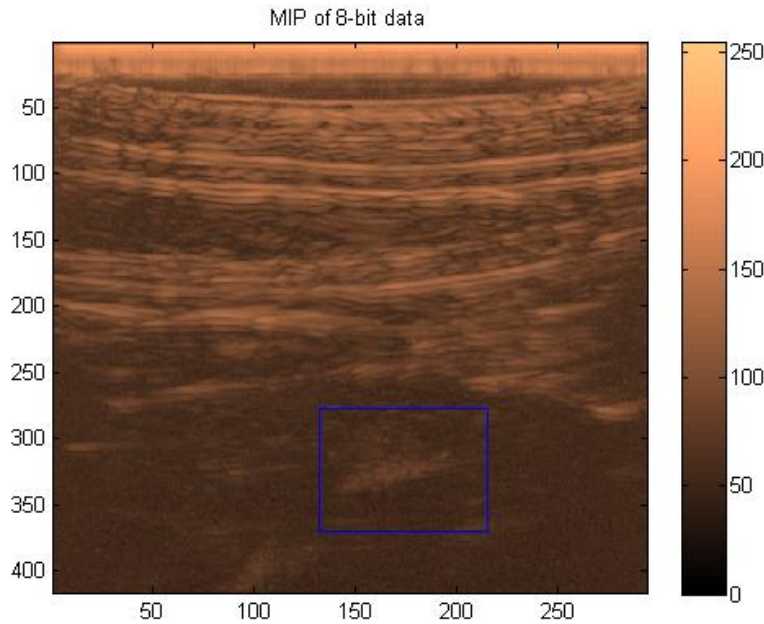
Our group has worked in partnership with GE to implement SHAPE on a state-of-the-art commercial scanner Logiq 9 with a mechanical 3D linear array (4D10L). Software to automatically optimize the acoustic output power for SHAPE has been developed on the scanner. Briefly, the optimization algorithm steps the ultrasound scanner from 0 to 100% output power [16]. A logistic equation fitting function was applied with the criterion of minimum least squared error between the fitted subharmonic amplitudes and the measured subharmonic amplitudes as a function of the output levels and the optimum level is chosen as the inflection point calculated from the fitted data. Likewise, software has also been developed to perform 3D SHAPE on the Logiq 9.

Several experiments have been carried out in canines to investigate 3D SHI and SHAPE for *in vivo* renal imaging and pressure estimation obtained with IV infusions of the microbubble based ultrasound contrast agent Definity (infusion rate: 3-10 ml/min for a 3 ml vial of Definity mixed with 50 ml of saline, which is the allowed range for human studies). For these optimization experiments, the dogs' kidneys were imaged (transcutaneously), because this is the most vascular organ and a good model for blood perfusion in tissue. We used regular imaging i.e., grayscale B-mode to confirm the appropriateness of the SHI imaging sites. We will also acquire simultaneous measurements of pressure in the kidneys with a Logiq 9 ultrasound scanner modified to obtain subharmonic microbubble signal amplitudes and with an intravenous 5F high-fidelity manometer-tipped pressure catheter (SPC-350; Millar Instruments, Houston, TX). The subharmonic and the pressure catheter data were compared.

## 5.2 Results and Discussion

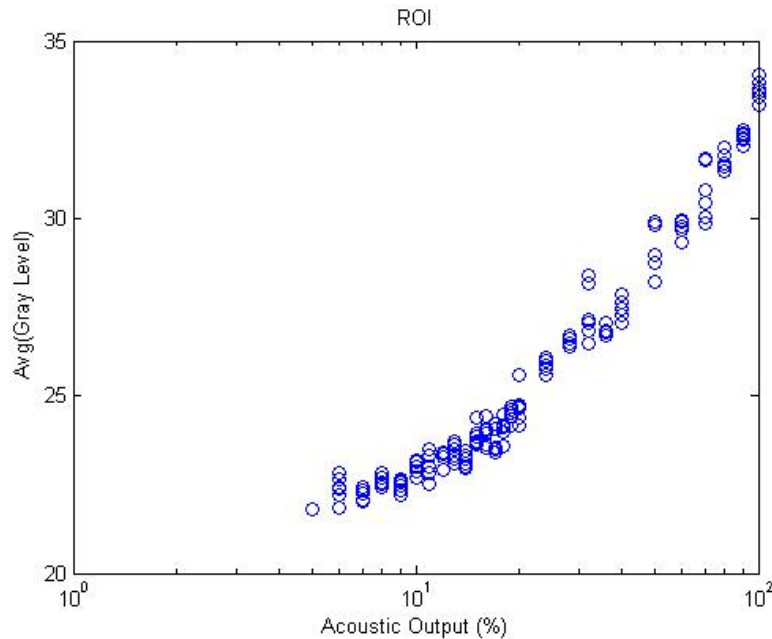
### *In vivo experiments*

Software has been developed and implemented on the Logiq 9 scanner to analyze RF data and produce SHAPE pressure estimates. To date, 2 canines have been imaged with good results to provide *in vivo* proof-of-concept of the feasibility of implementing 3D SHAPE on a commercial scanner. An example of the SHI images obtained in maximum intensity projection (MIP) mode (as part of the optimization algorithm) can be seen in Figure 1 for the left kidney. The corresponding curve showing the change in subharmonic amplitude as a function of acoustic power is shown in Figure 2. This effort represents the commencement of task 2.



**Figure 1.** MIP subharmonic data with a renal ROI marked (in blue) over a contrast enhanced blood vessel.

Further developments are ongoing and currently the main focus is on establishing the best method to transfer the subharmonic signal components between the different software applications running on the Logiq 9 scanner for this project.



**Figure 2.** The subharmonic amplitude as a function of acoustic power from within the renal ROI marked in Figure 1. Five images were analyzed at each power setting.

## 6 KEY RESEARCH ACCOMPLISHMENTS

- The regulatory requirements for conducting this clinical trial are almost complete.
- Software for processing of *in vivo* 3D SHAPE data directly on the Logiq 9 scanner has been developed.
- SHAPE *in vivo* experiments were conducted in canines with Definity using the Logiq 9 scanner and the 4D10L probe.

## 7 REPORTABLE OUTCOMES

### *Presentations*

May 21 – 23, 2012

The Leading Edge in Diagnostic Ultrasound, Atlantic City, NJ, USA.

- Monitoring neoadjuvant chemotherapy with subharmonic pressure estimates.

October 3 – 4, 2013

28<sup>th</sup> Annual Advances in Contrast Ultrasound & ICUS Bubble Conference, Chicago, IL, USA.

- Monitoring interstitial fluid pressure in breast cancer with subharmonics.

## 8 CONCLUSIONS

The development and implementation of 3D SHI and SHAPE software on the Logiq 9 scanner has been accomplished and optimization studies in animals are almost complete. However, the efforts required to obtain approvals for both animal and human trials were markedly more cumbersome than envisaged in the original submission. Nonetheless the necessary paperwork is almost completed and we expect to start the human clinical trial within the month of November.

In summary, task 1 has been partially completed while task 2 is ongoing, but due to the delay caused by the efforts required to obtain regulatory approval for the animal and human clinical trials, the project is approximately 8 months behind schedule and we, therefore, intend to request a one year no cost extension.

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## Appendix I

The Statement of Work from the original proposal:

### Objective 1

**Task 1:** Design and implementation of SHAPE on a commercial US scanner (months 1 - 4)

- a. Optimize 3D SHI and SHAPE based on the parameters associated with the designated transducer; the 4D10L (month 1).
- b. Modify a state-of-the-art US imaging system (the Logiq 9) to perform 3D SHAPE (since the 3D SHI contrast imaging modality was already incorporated on this system as part of R01 CA140338; months 1 - 3).
- c. Evaluate the 3D SHI imaging modality and 3D SHAPE in an *in vitro* flow phantom using the modified US scanner (month 3).
- d. Prepare regulatory submissions for clinical studies and obtain institutional approval for animal studies (month 2 - 4).

### Objective 2

**Task 2:** Optimize and calibrate *in vivo* 3D SHI and SHAPE in animals (months 4 – 5)

- a. Calibrate *in vivo* 3D SHAPE results based on pressure measurements obtained with a manometer-tipped pressure catheter (as the reference standard) in 3 mongrel dogs (months 4 - 5).
- b. Evaluate the ability of 3D SHI to depict macro- and micro-vascularity (the latter as a model for tumor angiogenesis) in the kidneys of 3 canines (months 4 - 5).

### Objectives 3 - 4

**Task 3:** Conduct human clinical trial, data collection and analysis (months 5 - 24)

- a. Validate the clinical potential of 3D SHI as a tool to monitor neoadjuvant chemotherapy (i.e., the ability to track changes in tumor angiogenesis) in women with LABC by studying 20 – 50 subjects before as well as after the 1<sup>st</sup>, 3<sup>rd</sup> and last chemotherapy cycle and comparing results to MRI and pathology findings (months 5 - 23).
- b. Validate the clinical potential of 3D SHAPE as a tool to monitor neoadjuvant chemotherapy (i.e., the ability to track changes in IFP) in women with LABC by studying 20 – 50 subjects before as well as after the 1<sup>st</sup>, 3<sup>rd</sup> and last chemotherapy cycle and comparing results to MRI and pathology (months 5 - 23).
- c. Evaluate the ability of 3D SHI to depict LABC neovascularity in women compared to CD31 stained specimens (Months 5 - 23).
- d. Perform statistical analyses and write final report (months 23 - 24).